Molecular surveillance of *Theileria* parasites of livestock in Oman

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Abstract

Background

Theileriosis is one of the most prevalent infectious diseases of livestock in the Arabian Peninsula, and causes high rates of mortality and morbidity in sheep and cattle. However, there is a paucity of information on the distribution of *Theileria* spp. over the whole region and their impact on different hosts. The present study carried out a country-wide molecular survey for *Theileria* spp. of livestock in Oman across four governorates. The aim of the survey was to define the prevalence of *Theileria* spp. in cattle, sheep and goats, highlight risk factors for infection and identify the main tick species involved in parasite transmission.

Material and Methods

A total of 2020 animals were examined in the survey consisting of sheep [n=592], goats [n= 981] and cattle [n= 447]. All three species were raised and co-grazed on the same farms. *Theileria* parasites were detected using PCR-RFLP and RLB of the 18S *rRNA* gene. Cloning and sequencing of the *18S rRNA* was carried out on 11 *T. lestoquardi* isolates from Ash-Sharqiyah, and Ad-Dhahira governorates, and phylogenetic relationships were inferred using additional sequences of *T. lestoquardi*, *T. annulata* and *T. ovis* available in GenBank.

Results

Theileria spp. prevalence was 72.3%, 36.7% and 2.7% among cattle, sheep and goats, respectively. Strong similarity in results was obtained using RLB and PCR-RFLP for detection of *Theileria* spp. however, RLB detected a higher rate of mixed species infection than PCR-RFPL ($P < 0.001$). *Theileria annulata* was the only parasite detected in cattle, while sheep and goats carried *T. ovis*, *T. lestoquardi* and *T. annulata* as well as *Theileria sp. OT1*. Of the four *Theileria* spp. detected in small ruminants, overall *T. ovis* was most prevalent (sheep [33.4%], goats [2.0%]), whereas *T. lestoquardi* was less prevalent (sheep [22.0%], goats [0.5%]). A large proportion of infected sheep (19%) carried mixed species infection of *T. ovis* and *T. lestoquardi*. However, single *T. lestoquardi* infections (3.0%) were less prevalent than *T. ovis* infections (14.5%). Risk of *Theileria* spp. infection was significantly higher for exotic breeds, relative to native breeds, of cattle ($p = 0.00002$) and sheep ($p = 0.005$). Phylogenetic analysis placed *T. lestoquardi* in Oman in the same clade as other *T. lestoquardi* strains isolated from the same regional area (Iraq and Iran). The main tick species, identified on the examined animals, *Hyalomma anatolicum*, was widely distributed and was found in all of the surveyed governorates.

Conclusion

Theileria spp. of parasite are widespread in Oman with variable prevalence detected in different regions. Two economically important hosts, cattle and sheep are at high risk from virulent *T. annulata* and *T. lestoquardi*, respectively. The survey indicates extensive exposure to ticks and transmission of infection that has a significant

economic impact. The higher prevalence of *T. lestoquardi* as mixed rather than single infection requires further investigation.

1. Introduction

Theileriosis is a complex parasitic disease of domestic ruminants caused by protozoan parasites of the genus *Theileria* and occurs primarily, but not exclusively, in tropical and subtropical regions (Heidarpour et al., 2009). The genus *Theileria* comprises more than 185 different species, with pathogenic species causing disease mainly in domestic ruminants and horses. *Theileria annulata* and *T. parva* are the most important pathogens of cattle, while *T. lestoquardi*, *T. luwenshuni* and *T. uilenbergi* are pathogenic to small ruminants (Panel and Ahaw 2010; Altay et al., 2012; Zaeemi et al., 2011).

Livestock represents an important economic and cultural heritage in the Arabian Peninsula. However, livestock sustainability and productivity is hampered by infectious disease of the region. Malignant theileriosis in cattle and or sheep has been recorded in Turkey (Sayin et al., 1997), Iran (Hashemi-Fesharki, 1997), Iraq (Latif et al., 1977), Saudi Arabia (Hussein et al., 1991; El-Metenawy., 1999) and Oman (Tageldin et al., 2005). In addition, theileriosis is also common in countries, such as Sudan (Taha et al., 2013), Ethiopia (Gebrekidan et al., 2014) and Somalia (Hassan et al., 2013) that have close livestock import/export links with Oman. Therefore, reducing mortality from theileriosis can be regarded as an important strategy to improve livestock productivity in Oman and other countries of the Arabian Peninsula. Limited surveys have demonstrated high susceptibility of Omani sheep breeds to *T. lestoquardi* (Tageldin et al., 2005, Al-Rubkhi, 2011). Many of these animals become sick and die before developing microschizonts and the intraerythrocytic piroplasm stage, and this may contribute to the failure to achieve early diagnosis of disease caused by this species (Tageldin et al., 2005; Shayan et al., 2011). However, Omani goats are more resistant to the disease, with a very low prevalence of *Theileria* parasites compared to sheep indicated (Tageldin et al., 2005). A pilot study in the centre of Oman (Ad-Dakhilia and Al-Batinah) showed that *T. ovis* and *T. lestoquardi* are the major causative agents of ovine theileriosis (Al-Weheibi, 2011). Interestingly, a large proportion of infected sheep (28%) were found to harbour mixed species infection of *T. ovis*, *T. lestoquardi* and *T. annulata*. No information is currently available on the extent of infection caused by pathogenic *Theileria* species among cattle in Oman and whether *Theileria* species from small ruminants can serve as a reservoir for infection of co-grazed cattle.

The aim of the present study was to identify *Theileria* species in ovine, bovine and caprine hosts using molecular assays, and determine the prevalence of *Theileria* spp. in four geographically distinct regions to examine the potential for differences with respect to both region and ruminant host. Moreover, the most common tick species in areas where theileriosis is endemic and potential risk factors for *Theileria* infection were assessed. In addition, the phylogenetic relationship between *T. lestoquardi* in

Oman and in different countries within the general geographical region was determined.

2. Materials and methods

2.1 Study area and animals

The study was conducted in four distinct governorates of Oman; Ash-Sharqiyah (East), Ad-Dhahira (West-north), Al-Batinah (North) and Dhofar (South) (Fig 1). A total of 2020 blood samples were collected from animals apparently clinically normal without history of theileriosis between April and August 2014, from the selected districts. Small ruminants were free ranging during the day and kept in confined areas (indoors) during the night. Cattle, excluding those in Dhofar, are zero-grazing animals and kept in-doors: in Dhofar cattle are allowed to graze during the day.

Four ml of blood was collected into EDTA tubes from randomly selected animals from each herd. Demographic data including age, gender and breed were recorded. Indigenous, cross and exotic breeds were included for each host: bovine, ovine and caprine. The exotic breeds were Friesian, Somali, Ethiopian and Pakistani.

2.2. PCR-RFLP analysis

DNA was extracted from 200 µl of blood using the Qiagen mini DNA extraction kit (Qiagen, Germany), according to the manufacturer's instructions. All 2020 samples were screened by PCR using the *Theileria* genus-specific primers, [forward 5'-GGC GTT TAT TAG ACC TAA AAC CAA AC-3' and reverse 5'-TTT GAG CAC TCT AAT CTC AAA GT-3'], with a single 530 bp fragment of the *18S rRNA* gene amplified for all *Theileria* species (Al-Hamidhi et al. 2015; 2016). PCR was followed by RFLP analysis of all positive samples to discriminate different *Theileria* species, as described by Heidarpour et al. (2009). Previously analysed DNA samples of *T. annulata*, *T. ovis* and *T. lestoquardi* were also included in each PCR reaction and RFLP analysis to act as standards for the assay, details for PCR conditions were as described (Heidarpour et al. 2009).

PCR products were digested with the restriction enzyme; *HpaII* (BioLabs, New England) according to the manufacturer's instructions. Digested PCR products were then run on 2.5 % agarose gel, stained with ethidium bromide. Expected sizes of the fragments obtained from the digested PCR product representing *T. annulata* were 357bp, 94 bp and 39 bp: for *T. lestoquardi* 276 bp, 88 bp, 79 bp and 39 bp and for *T. ovis* 326 bp 136 bp, 39 bp and 35 bp (Heidarpour Bami et al., 2009).

2.3. RLB hybridization assay for the detection of *Theileria/Babesia* species in collected samples

RLB hybridization of the 18S amplicon was performed as previously described (Gubbels et al., 1999; Schnittger et al. 2004) with the following modification. For the amplification of the V4 hypervariable region of the 18S Ribosomal RNA (rRNA) gene

of *Theileria* species the forward primer used was RLB-F2 (5′-GACACAGGGAGGTAGTGACAAG) and the reverse primer was RLB-R2 (biotin-5′-CTAAGAATTTCACCTCTGACAGT) as described by (Oura et al., 2003). PCR reactions were performed in 50 µl, containing 1 x PCR buffer (Thermo Scientific Corp.), 1.5 mM $MgCl_2$ (Promega, Madison, WI, USA), 200 mM of each dNTP, 2.5 U of hotstart *Taq* polymerase (Thermo Scientific Corp.), 25 pmol each of forward and primers, and 2 µl of template DNA.The temperture profile for the PCR reactions were as described (Gubbels et al., 1999; Schnittger et al. 2004)

Genus and species, specific 18S rRNA oligonucleotides with an N-terminal N-trifluoracctamidohexyl-cyanocthyl, N, N-diisopropyl phosphoamidite [TFA]-C_6 amino linker were immobilised on a Biodyne-C nitrocellulose membrane (Gelman Lab., Pall Corp., United States). Oligonucleotide probes used to detect *Theileria/Babesia* species: *Theileria* and *Babesia* catch all, *Theileria spp.* catch all, *Theileria* species, specific (*T. ovis*, *T. lestoquardi*, *T. annulata*, *T. uilenbergi*, *T. luwenshunni*, *Theileria spp. OT1*, *T. spp. TO3*, *T. spp. MK*, *T. sperata)*, *Babesia spp.* catch all, *Babesia* species, specific (*B. ovis*, *B. m3*, *B. m2-2*, *B. m1*, *B. motasi*, *B. cG*, *B. cI*, *B. cT*)) were as described (Gubbels et al., 1999; Schnittger et al., 2004). The oligonucleotides were diluted to previously optimized concentrations ranging from 50 to 300 pmol in 150 µl 500 mM NaHCO3 (pH; 8.4). Probes were covalently linked to the Biodyne-C membrane as described by Schnittger et al. (2004). For hybridisation, 15 µl of biotin-labelled PCR products were diluted in 2xSSPE/% 0.1 SDS solution to a total volume of 150 µl. Diluted PCR products were then heated to 99°C for 10 min and cooled on ice, immediately. Manifold slots were filled with the denatured PCR products and hybridization preformed at 42 ^{O}C for 1 hour. Membranes were then washed and signal developed as described (Schnittger et al. 2004). Each membrane was reused up to 12 times.

2.4 Sequence and phylogenetic analysis of *T. lestoquardi 18S rRNA* gene

A total of 11 randomly samples of *T. lestoquardi* from Ash-Sharqiyah and Ad-Dhahira were selected for sequence analysis of the *18S rRNA* gene. PCR amplification, cloning and sequencing were carried out as described previously (George et al., 2015). Alignment and analysis of the *18S rRNA* sequences was performed using BioEdit version 7.2.5 software (Hall, 1999) to identify novel SNPs using the available sequence derived from Iran vaccine strain [GenBank: EU915292.1] as the reference.

Sequences of *T. lestoquardi 18S rRNA* gene were used to construct a tree using MEGA 6 (Tamura et al., 2013), in order to trace the predicted phylogenetic relationship between samples from Oman and additional sequences representing isolates from Arabia, retrieved from GenBank (accession #: GU726902, KJ024367, AF081135, EU915292). In addition, *T. annulata* sequences (accession #: AY260171-72, AY533144, FJ603460) and *T. ovis* sequences from Sudan, Turkey, Spain and China (accession #: KF429800.1, M64243.1, AY524666.1, KF42799.1, KF429793.1)

obtained from gene bank were included in the analyses. Phylogenetic trees were constructed using maximum likelihood (ML) in the MEGA 6 program. To estimate the reproducibility of the tree, bootstrapping was performed by tree reconstruction of random draws of sub-samples 1000 times.

2.5 Identification of tick species

Ticks were removed from infested cattle (n=39), sheep (n=17) and goats (n=16) in Ad-Dhahira and Ash-Sharqiyah governorates of Oman and collected in small labelled plastic containers containing 70% ethanol. Tick species were then identified microscopically according to Hoogstraal (1956) and Walker et al., (2003).

2.6 Statistical analyses

The effect of age, gender and breed as risk factors for *Theileria* infection in Oman was examined using binomial logistic regression analysis using the Statistical Package for the Social Sciences (SPSS) program version 19. Reference variables were as follows: for age group, less than one year old; gender, female and breed, indigenous (Salih et al., 2007). The program reports an odds ratio (OR) value of one for reference variables and if the tested variable has an OR of less than one a lower risk is indicated, if greater than one, elevated risk is indicated and if it more than two, a high risk is predicted for the factor.

3. Results

3.1 Overall prevalence of *Theileria* parasite in Oman

A total of 2020 domestic ruminants including 447 cattle, 595 sheep and 981 goats, from four governorates in Oman, were assayed for infection with *Theileria* spp. (Fig 1). The overall prevalence of infection with *Theileria* spp. among all hosts was 28% (566/2020). The prevalence varied significantly ($P < 0.05$) between different hosts, being higher for cattle (72.3% [323/447]) compared to sheep (36.7% [217/592]), and was found to be relatively rare in goats (2.7% [26/981]) (Table 1).

3.2 *Theileria* infection in different geographic locations

The overall prevalence of *Theileria* spp. differed significantly ($P < 0.05$) between the four governorates; being higher in Dhofar 36.1% (173/479), followed by Ad-Dhahira 30.6% (145/474), Ash-Sharqiyah 28.6% (116/405) and Al-Batinah 19.9% (132/662) (Fig 1). In Dhofar, 80.3 % of cattle were infected, all carrying *T. annulata*, while infection with any species in sheep (1.9 %) and goats (0.5 %) was negligible compared to cattle. In Al-Batinah there was a significant ($P < 0.001$) difference in infection rate between cattle (74.4%) and sheep (28.2%), while goats were not infected. In Ad-Dhahira 56.7%, 52.5% and 1.9% of cattle, sheep and goats were infected, respectively, with no significant differences between cattle and sheep ($P=0.294$). In Ash-Sharqiyah, the infection rates were 72.2%, 50% and 9.1% of cattle, sheep and goats, respectively, with significant differences in the infection between the three hosts ($p<0.05$). The

infection rate among goats was significantly higher in Ash-Sharqiyah compared to other governorates (P<0.001) (Fig 1).

3.3 Comparison between RLB and PCR-RFLP in detection of *Theileria* spp.

The agreement between RLB and PCR-RFLP for detection of *Theileria* spp. was found to be high (95%). Out of 1090 samples examined by both methods, 905 (83.0%) were negative for both tests while 134 (12.3%) were positive for both tests (Table 2).

For species identification, the agreement between the two tests was 91.9 %. RLB detected infection rates of 1.1%, 7.3%, 0.6% and 7.2% and PCR-RFLP revealed 3.4%, 5.5%, 0.7% and 3.2% of *T. lestoquardi*, *T. ovis*, *T. annulata* and mixed species, respectively. There was a substantial agreement between PCR-RFLP and RLB test results in detection of *Theileria* species, Cohen's kappa (κ) test, κ -value = 0.695 (Table 2). However, RLB detected significantly (P < 0.001) higher prevalence of mixed species infection compared to PCR-RFLP. Indeed, 29 samples detected as single infections of *T. lestoquardi* by PCR-RFLP were found to carry both *T. lestoquardi* and *T. ovis* by RLB.

3.4 Mixed species infection

Mixed species infection was common among small ruminants surveyed, particularly sheep; however, it was not detected for cattle, where only *T. annulata* positive animals were detected. Approximately 19% of the examined sheep carried mixed infection of *T. ovis* and *T. lestoquardi*, while infection with *T. lestoquardi*, *T. ovis* and *Theileria sp. OT1* constituted 0.3% (Table 2). Only, a small proportion of goats carried mixed species infection (0.5%, [5/981]) (Table 1).

3.5 Host related risk factors associated with *Theileria* infection

A total of six bovine, four ovine and four caprine breeds were examined in the survey. Breeds were grouped as indigenous (Omani and Dhofari), cross-breed (Omani with Friesian and Omani with Najdi) and exotic (Somali, Friesian, Ethiopian and Pakistani). Exotic ovine and bovine breeds showed a significantly higher risk for *Theileria* infection (OR of *Theileria* infection of 7.07 (p = 0.005) and 3.63 (p= 0.02), respectively) compared to the indigenous breeds. In addition, an OR of 2.04 was reported for cross breeds of bovine that was found to be significantly different (p = 0.045) to the infection risk for indigenous breeds. However, no significant difference in infection rate was seen between caprine breeds (Table 3).

This study examined 97 males versus 496 female sheep, 114 males versus 867 female goats and 61 males versus 250 female cattle. An odds ratio of more than one was reported for male hosts in ovine, caprine and bovine (1.56, 1.88 and 1.27 respectively); however, these ratios were not significantly different (p = 0.105, p = 0.27 and p = 0.52 respectively) compared to female animals (Table 3).

There was no statistically significant difference in the prevalence of *Theileria* spp. infection between different age groups of ovine, caprine and bovine hosts. However, in bovines an infection pattern was observed, as the prevalence of *Theileria* positives increased with age. The infection prevalence starts high in calves (<1 yr) and increased until the second year of age, after which a slight decrease was observed. Then from the third year and onward the prevalence of infection again increased with age (Table 3).

3.6 Phylogenetic analysis of *T. lestoquardi*

Analyses of 11 sequences revealed 5 SNPs within the 695 bp of the *18S rRNA* gene. Thus, 5 distinct *T. lestoquardi* sequences among Omani isolates were obtained, suggesting five different genotypes.

The maximum-likelihood phylogenetic tree showed that *T. lestoquardi* in Oman falls into the same clade as *T. lestoquardi* from other countries in the region (Iraq and Iran). As expected, *18S rRNA* sequences of *T. lestoquardi*, *T. annulata* and *T. ovis* fall into different clades. However, *T. lestoquardi* and *T. annulata* were found to show a close phylogenetic relationship, with a common ancestor predicted, and showed divergence from the *T. ovis* lineage (isolates from Sudan, Turkey, Spain and China) (Fig 2).

3.7 Tick species

To identify the main tick species present in the survey sites, 249 ticks were collected on animals in two governorates: 155 were on cattle, 52 on sheep and 42 on goats; indicating a tick load of 3.9, 3 and 2.6 respectively. Of the total collected, 246 were adult ticks (113 males, 133 females) and 3 were nymphs. Ninety eight percent of the adult ticks were *Hyalomma anatolicum (H. anatolicum)*. Only one tick of *H. excavatum* (atypical specimen) was detected on a bovine, and one female *Rhipicephalus guillhoni* was collected from a caprine host. All of the collected nymphs were of *Hyalomma* spp. and no larvae were found. Thus, the main tick species is *Hyalomma anatolicum* and this species is widely spread in the sampled governorates.

4. Discussion

Four *Theileria* species were identified in Oman, *T. annulata*, *T. lestoquardi, T. ovis* and *Theileria sp. OT1*. Cattle were infected only with *T. annulata,* while small ruminants were infected with, *T. ovis, T. lestoquardi*, *T. annulata* and *Theileria sp. OT1*. Two species, *T. ovis* and *T. lestoquardi* were detected at highest prevalence among small ruminants; *T. lestoquardi* is known to be the main causative agent of ovine theileriosis in Oman (Tageldin et al., 2005), while *T. ovis* is the dominant *Theileria* species in terms of prevalence. A high proportion of sheep carried mixed infections of *T. ovis* and *T. lestoquardi*. This study represents the first systematic molecular survey of *Theileria* spp. in the Gulf Co-operation Council countries (GCC, consisting of Qatar, the UAE, Kuwait, Bahrain, Saudi Arabia and Oman), highlighting a high risk of livestock to infection by these tick borne parasites.

The presence of *Theileria* spp. in small ruminants in Oman is consistent with that seen in other countries in the middle east and neighbouring countries, Iraq, Pakistan and Iran (Oliveira et al., 1995; Al-Saeed et al., 2010; Khan et al., 2013). However, in Oman *T. ovis* (10.7%) and *T. lestoquardi* (6.6%) were less prevalent than in other countries such as Pakistan (*T. lestoquardi* [21%], *T. ovis* [79%]) (Durrani et al., 2011; Iqbal et al., 2013), and Sudan, (*T. lestoquardi* [16.3%], *T. ovis* [88.6%]) (Elimam, 2010). This regional variation in distribution of *T. lestoquardi* and *T. ovis* agrees with the differing global pattern in prevalence of *Theileria* spp. For example, in Turkey *T. lestoquardi* has not been detected, while *T. ovis* (34.6%) was common (Altay et al., 2005., 2007, 2012; Aydin et al., 2015, 2013). The differences in distribution of *Theileria* spp. in different areas can be explained by variation in environmental conditions, tick vector abundance, different managements systems and genetic differences in susceptibility between available ruminant hosts (Chaussepied et al., 2010). Any of these factors could influence the observed heterogeneity in *Theileria* infectivity in different governorates in Oman. Differences were also observed between farms within the same governorate (data not shown) and further surveys controlling for breed differences, and farm management etc. would need to be performed to fully understand regional variation in greater detail.

Theileria annulata, which is commonly found in cattle and considered to be highly pathogenic, was detected in seven small ruminants (1 ovine and 6 caprine). Since cattle, sheep and goats are raised together on the same farms, it is feasible to propose that while cattle can be a reservoir for ovine infection, small ruminants are less susceptible to *T. annulata* than cattle. Similar findings were reported in Iran, where, *T. annulata* was detected in sheep mixed with other species, *T. lestoquardi* or *T. ovis* (Zaeemi et al., 2011; Jalali et al., 2014), in the Sudan, where 7.8% of infected sheep carried mixed infection of *T. annulata* and *T. lestoquardi* (Taha et al., 2013) and in Turkey (Aktas and Ozubek, 2015). Moreover, antibodies against *T. annulata* have been detected in sera of naturally infected sheep (Salih et al., 2003). Experimental transmission of *T. annulata* infected cell lines into sheep and goats has been demonstrated to cause clear symptomatology in sheep but only mild symptoms in goats (Brown et al., 1998). Additionally, it has been reported that *T. annulata* sporozoites can infect sheep and cause mild clinical signs with the appearance of schizonts in infected animals, although no piroplasms were observed. This observation indicates that the life cycle of *T. annulata* is incomplete in sheep (Leemans et al., 1999), and likely plays no role in maintaining a ovine-tick-ovine *T. annulata* infection cycle in the field (Li-jun et al., 2013).

In the current study, the prevalence of mixed infection with *T. lestoquardi* and *T. ovis* in sheep (18.5% [110/592]) was higher than single infection of *T. ovis* (14.5%[86/592]) or *T. lestoquardi* (3% [18/592]) (p-value < 0.01). However, in Iran, mixed infection of these two species were detected at a lower prevalence of 6.6% than single species infections (Rashidi and Razmi, 2013). Mixed species infection is a common feature of protozoan and rickettsial pathogens in nature, including, *Theileria*

(Rashidi and Razmi, 2013), *Babesia* (Aktas and Ozubek, 2015), *Plasmodium* (Cantas et al., 2013), *Trypanosoma* (Gillingwater et al., 2010) and *Anaplasma* (Aktas et al., 2011; Aktas and Özübek, 2015). It is not known whether multiple parasite species infection is driven by environmental, parasite or host factors. However, experimental data in sheep indicate that *T. lestoquardi* can protect against subsequent *T. annulata* infection (Leemans et al., 1999) and *T. annulata* can protect against the major clinical effects of *T. lestoquardi* infection (Leemans et al., 1999). Moreover a recent study has suggested that the pathological effect of *T. parva* is mitigated by the presence of less pathogenic *Theileria* spp. resulting in substantial reduction in the risk of morbidity and mortality due to co-infection by congeneric parasites (Woolhouse et al., 2015). Whether such a scenario also operates in ovine *Theileria* infection, with *T. ovis* providing protection against clinical signs mediated by the more pathogenic *T. lestoquardi* requires further study, including investigation of clinically infected animals for single or mixed species infection

A previous phylogenetic analysis of *T. lestoquardi* suggests that isolates derived from the Al-Batinha governorate in Oman are relatively distinct from isolates from Sudan and Iran that are known to be pathogenic (Al-Rubkhi, 2011). However, the present study revealed close relationship between *T. lestoquardi* in Ash-Sharqiyah and Ad-Dhahira and other *T. lestoquardi* strains in the region (Iraq and Iran). This is consistent with a of regional separation based on *18S rRNA* sequences of *T. annulata* in Iran and other countries in the region, Iraq and Turkey (Habibi, 2013). The differences between the findings of the present study and those of Al-Rubkhi (2011) could be due to the fact that sheep in Al-Batinha are geographically isolated from those in Ash-Sharqiyah and Ad- Dhahira. Thus, further analysis of *T. lestoquardi* in Al-Batinha is needed to verify this observation.

Theileria sp. OT1 was detected in three small ruminants Omani breeds from Ash-Sharqiyah governorate together with *T. ovis* and *T. lestoquardi*. This is the first report of this species in Oman. There is currently not much information about the pathogenicity of *Theileria sp. OT1*, but it has been linked to the pathogenic *Theileria sp.* China 1 (Altay et al., 2012; Nagore et al., 2004). However, it has also been suggested that *Theileria sp. OT1* is not a pathogenic species, since it was found in asymptomatic animals and does not alter red blood cell parameters (Nagore et al., 2004). Further work is required to establish any clinical impact of this species on ovines in Oman.

The most common risk factors associated with *Theileria* spp. infection in Oman were found to be host type (cattle, sheep or goats) and breed. Cattle and sheep were highly susceptible to *Theileria* infection, while goats appeared to be less so, although regional variation in goat infectivity was detected. The overall rate of infectivity among goats was 2.7% comapred to 36.7% and 72.3% in sheep and cattle, respectively. This agrees with the fidings of many studies in other regions (Altay et al., 2007; Gebrekidan et al., 2014). For example, in Turkey the prevalence of *Theileria* spp. among goats (11.27 %) was much lower than in sheep and cattle (58.79%) in the same area (Altay et al.,

2007). This has been attributed to the nature of the skin of goats, which is more resistant to tick attachment compared to sheep (Fatima et al., 2015). It has also been hypothesised that sporozoites of *T. parva* are not able to easily invade caprine lymphocytes (Syfrig et al., 1998), but whether this applies to *T. annulata* and the ovine *Theileria* species is not known. Further studeis are needed to examine attributes of low suscepotibility of goats in Oman to *Theileria* spp.

Exotic breeds of bovine and ovine are highly susceptible to *Theileria* spp. compared to indigenous and cross breeds. Indigenous breeds are known to have a natural ability to develop higher levels of resistance to tick borne diseases (TBDs) compared to cross and exotic breeds (Gebrekidan et al., 2014; Salih et al., 2007). This can lead to clearance of infection or a reduction in the parasite load and this might explain why indigenous breeds showed a lower *Theileria* spp. infection prevalence in comparison to exotic breeds in our study. These results are consistent with the findings of a study in Sudan which reported that *T. annulata* infection was 70% lower among the local breed (kenana) compared to the non-local (Friesian) (Bakheit and Latif, 2002).

In summary, this study demonstrated a widespread distribution of *Theileria* spp. among domestic ruminants in Oman with exotic breeds of cattle and sheep more susceptible to *Theileria* infection than local breeds. Further studies are required to investigate the cost benefit of native breed resistance versus exotic breed productivity gain and the impact of mixed species infection on manifestation of clinical disease. This data would be of benefit in formulation of a national control strategy for theileriosis and other tick borne diseases of domestic animals in Oman.

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Table 1: Distribution of *Theileria* species in ovine, caprine and bovine hosts across four studied governorates.

		Ovine n = 592	Caprine n = 981	Bovine n = 447	Total n= 2020
	Positive cases (%)	217 (36.7)	26 (2.7)	323(72.3)	566 (28)
Single Infection	*T. lestoquardi*	18 (3.0)	-	-	18 (0.89)
	T. ovis	86 (14.5)	15 (1.5)	-	101 (5)
	T. annulata	1 (0.2)	6 (0.6)	323(72.3)	330 (16.3)
Mixed Infection	*T. lestoquardi/ T. ovis*	110(18.5)	4 (0.4)	-	114 (5.6)
	T. *lestoquardi/ T. ovis/T. sp* OT1	2 (0.3)	1(0.1)	-	3 (0.15)

n; number of samples

Table 2: Comparison of PCR-RFLP and RLB assays in the detection for *Theileria* spp. in 1090 blood samples of small ruminants in Oman.

		RLB					Total
		negative	*T. lestoquardi*	*T. ovis*	*T. annulata*	Mixed*	No.(%)
PCR-RFLP	negative	905	4	30	0	9	948 (86.9)
	T. lestoquardi	2	7	0	0	29	38 (3.4)
	T. ovis	5	1	49	0	6	61 (5.6)
	T. annulata	0	0	1	7	0	8 (0.7)
	Mixed*	1	0	0	0	34	35 (3.2)
Total No. (%)		913 (83.7)	12 (1.1)	80 (7.3)	7 (0.6)	78(7.2)	1090

*Mixed: mixed species with *T. ovis* and *T. annulata*. No: number of samples. Cells highlighted with dark-grey: negative results, light-grey: number of samples that have same result from both tests. Underlined blue numbers: number of samples which show a different result with each of the test. The percentages were calculated out of total samples (1090).

Table 3: Odds ratio of the risk factors of *Theileria spp.* infection obtained from logistic regression analysis

Risk factors	Level	Ovine		Caprine		Bovine	
		OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Breeds	Indigenous	1		1		1	
	Cross	0.89 (0.56 -1.41)	0.608	-	-	2.04 (1.02- 4.09)	0.045
	Exotic	7.07 (1.80 – 27.79)	0.005	0.00	0.998	3.63 (1.22-10.8)	0.020
Gender	Female	1		1		1	
	Male	1.56 (0.91- 2.65)	0.105	1.88 (0.61- 5.79)	0.272	1.27 (0.61- 2.66)	0.520
Age groups	>1 year	1		1		1	
	1-2 years	1.02 (0.59- 1.79)	0.935	2.37 (0.619.22)	0.213	2.02 (0.69-5.95)	0.203
	2-3 years	1.34 (0.78- 2.32)	0.294	1.82 (0.45-7.32)	0.401	0.82 (0.371.80)	0.620
	3-4 years	1.29 (0.71- 2.36)	0.401	0.62 (0.10-3.82)	0.602	1.36 (0.613.02)	0.456
	4-5 years	2.30 (1.05- 5.04)	0.037	1.09 (0.18-6.86)	0.924	1.88 (0.794.51)	0.155
	>5 years	1.12 (0.45- 2.82)	0.806	2.84 (0.55-14.7)	0.212	2.43 (1.10-5.35)	0.028

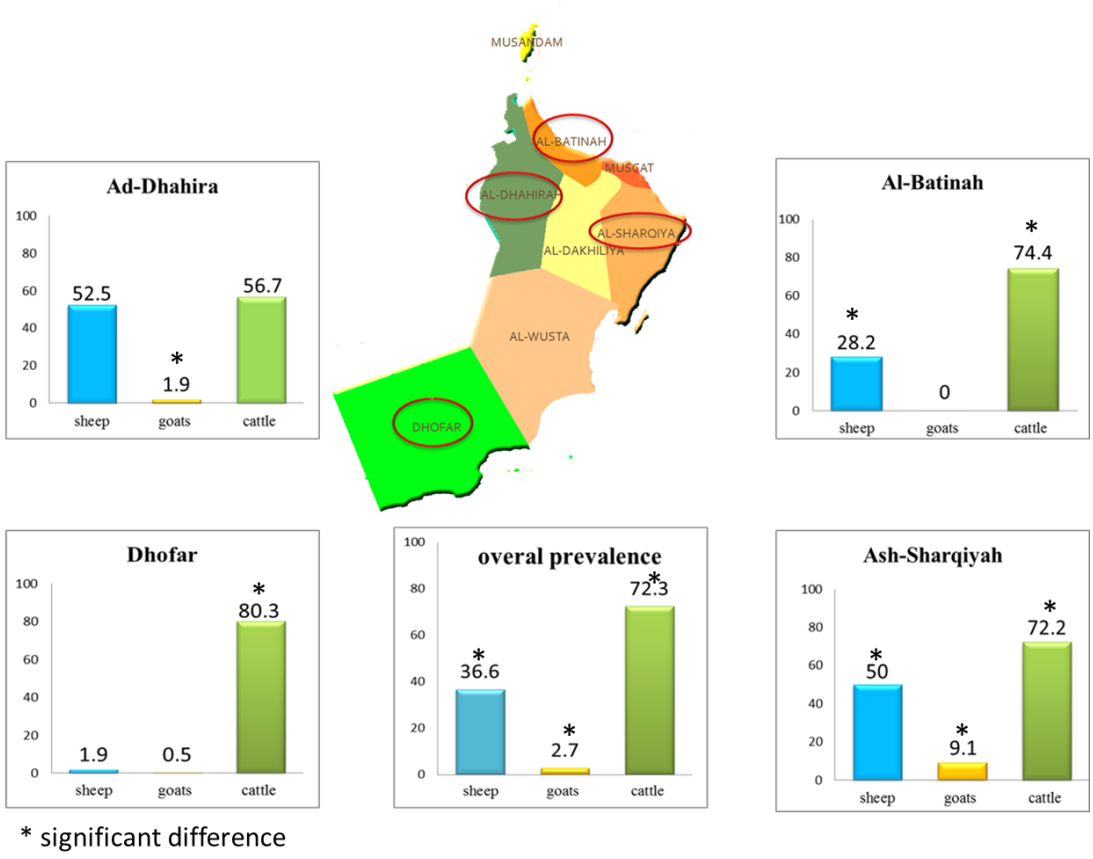

Fig 1: Prevalence of *Theileria* spp. among ovine, caprine and bovine hosts in four governorates in Oman

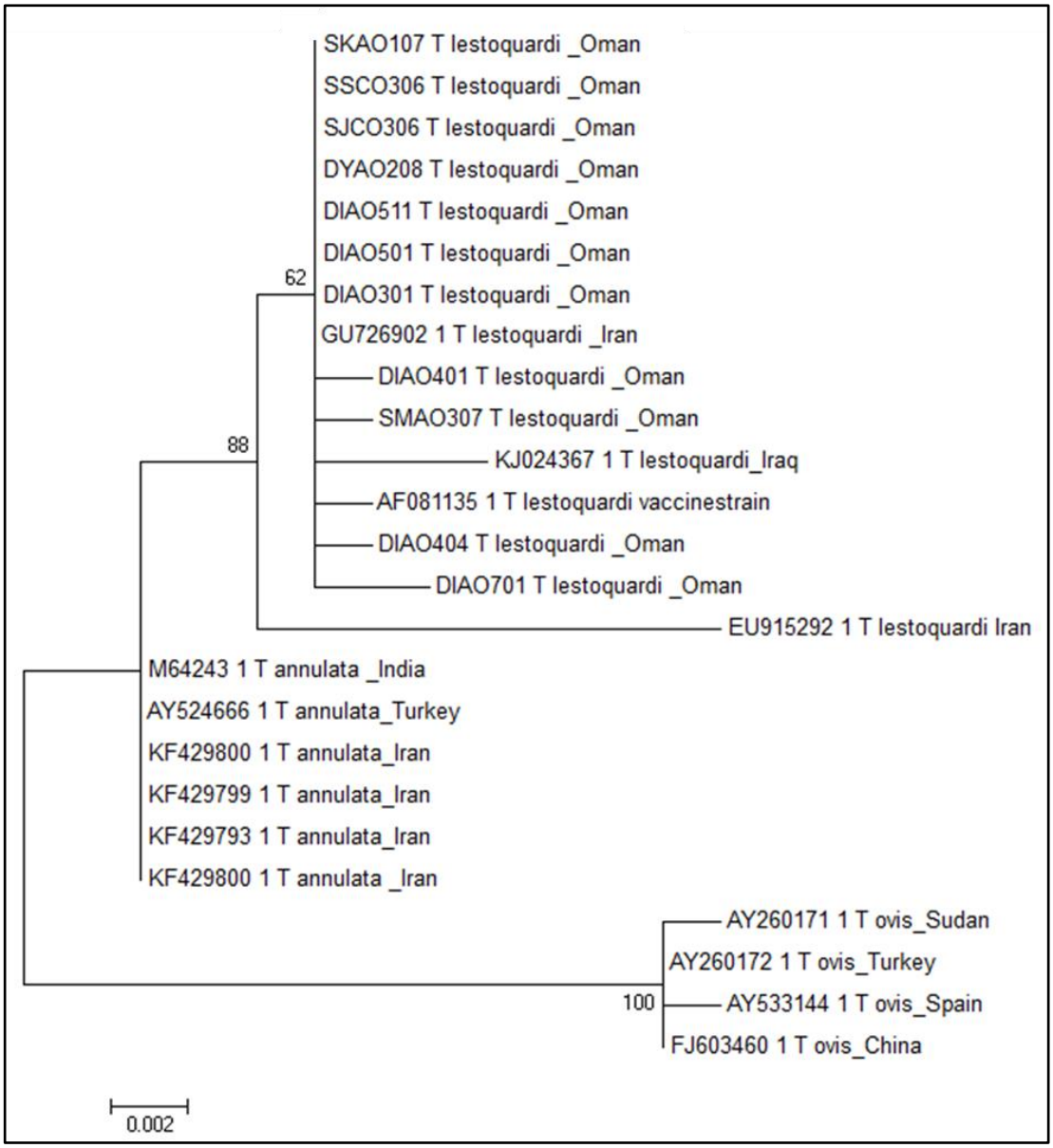

Fig **2**: Phylogenetic tree constructed using *18s rRNA* gene sequences of *T. lestoquardi*, *T. annulata* and *T. ovis*. The phylogenetic tree was constructed using the Maximum Likelihood method based on the Jukes-Cantor model in MEGA 6 program. The accession number of number of available reference sequences is indicated.

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